Ecological Indicators 98 (2019) 694–703

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

# Rainfall variation shifts habitat suitability for seedling establishment associated with tidal inundation in salt marshes

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ARTICLE INFO

*Keywords:*
Niche
Plant–soil interactions
Seedling emergence
Soil salinity
Soil moisture
Species composition
Spring rainfall

ABSTRACT

Growing concerns over the ecological consequences of global changes in rainfall regimes have motivated exploration into how variable rainfall influences the persistence of plant species. Understanding the influence of rainfall events on seedling establishment is critical for predicting the consequences of potential changes to the rainfall regime in coastal salt marshes. However, it is not clear whether seedling establishment of various species responses to rainfall are strongly regulated by tidal inundation. We conducted a series of rainfall manipulation experiments in salt marshes and controlled greenhouse experiments to test the effect of post-dry season rainfall on seedling establishment of plant species among different tidal elevations and during drought and large rainfall events. Our results showed that post-dry season rainfall created an opportunity for seedling establishment by strongly influencing the suitability of abiotic factors for species niches, but plant–soil–rainfall interactions were nonlinear across existing soil conditions, which were controlled by tidal inundation. Seedling establishment was highly dependent on post-dry season rainfall amount. The seedlings of species that were sensitive to saline soil or had low tolerance to soil moisture only established successfully during large rainfall events. Consequently, changes in the rainfall amount caused shifts in native species composition and opened invasion opportunities for new species. Our study highlighted that small changes in rainfall amount or distribution can trigger comparatively large and abrupt changes in different species depending on their ecological niches and local abiotic gradients. Within tidal marshes, exploration of rainfall–tide linkages advanced fundamental understanding of the effects of changing rainfall regimes and provided implications for coastal wetland management and restoration efforts to prepare for the future rainfall changes related to climate change.

## 1. Introduction

Global climate changing is expected to perturb both total rainfall amount and intra-annual distribution of rainfall (Goldstein and Suding, 2014). With the growing investigation to rainfall regimes from recent empirical and model studies, what is becoming clearer is that the rainfall may have strong effects on plant population persistence (HilleRisLambers et al., 2001; Hulme, 2005; Goldstein and Suding, 2014; Yando et al., 2016). The extreme rainfall event (Jentsch et al., 2007; Smith, 2011a,b) or severe drought (He et al., 2017) may result in rapid degradation of plant species. Problem is worst for annual plants, which must germinate and establish sufficient seedlings every year (NOE and Zedler, 2001) to persist in habitats. Germination niche is considered one of the most vulnerable thresholds in the life cycle of plants (Smith et al., 2000; Zimmermann et al., 2008). The intra-annual rainfall may have particularly strong effects on moisture or water-related abiotic gradient (NOE and Zedler, 2001) and results in big changes in suitability of abiotic factors for seedling niches. Therefore, challenges for advancing understanding of the ecological consequences of global rainfall pattern changing on plant persistence consists in identifying the interaction of thresholds of abiotic factors changing due to rainfall effect with species niches, such as germination niche in various ecosystems (Smith, 2011a,b).

The effect of rainfall is particularly important in coastal salt marshes where freshwater limitation determines the survival of plant species (Ewel, 2010; Osland et al., 2016). Studies have shown that in salt marshes, soil salinity and moisture levels are the largest factors regulating halophyte seedling establishment (Davis, 1991; Pennings and Callaway, 1992; Levine et al., 2011; Ozfidan-Konakci et al., 2016). The most difficult plant–soil interactions occurring in salt marshes

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https://doi.org/10.1016/j.ecolind.2018.11.056
Received 11 September 2018; Received in revised form 21 November 2018; Accepted 22 November 2018
Available online 26 November 2018
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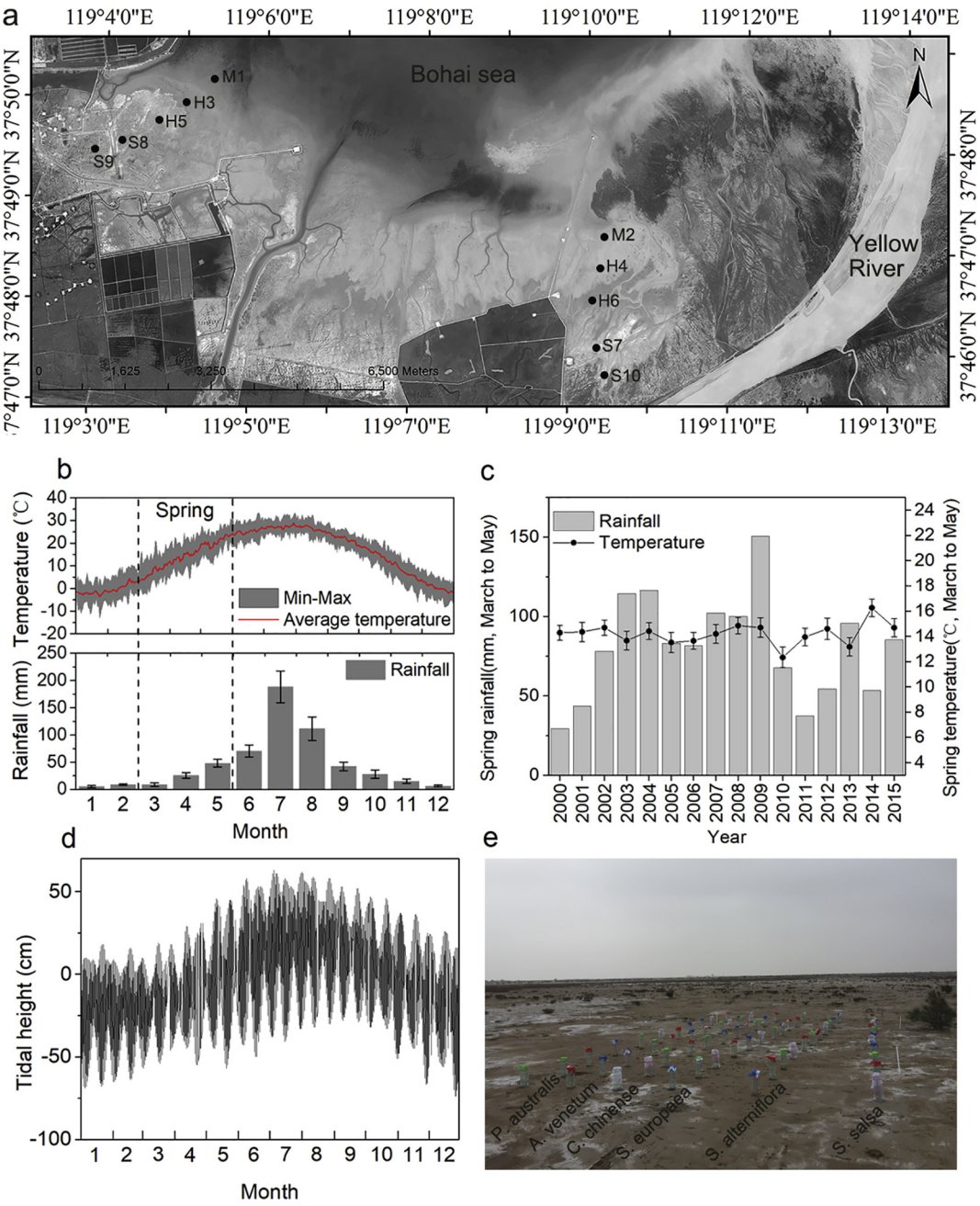


**Fig. 1.** (a) Map of the study sites. The study area was mapped using ArcGIS 10.3 software (Esri China Information Technology Co. Ltd, Hong Kong, China, http://www.esri.com/) based on the remote sensing image data purchased from SPOT 3. (b) Mean monthly rainfall and daily temperature during 2000–2015, measured at China's national ground meteorological station (Lijin weather station, 118°33′E, 37°35′N) and Dongying Hydrological Administration (http://dyswj.sdwr.gov.cn/). (c) The spring rainfall and average temperature during 2000–2015. (d) Tide levels in 2015 were estimated based on the astronomic tide model at the Dongyinggang tidal station (118°58′E, 38°06′N). (e) The photograph of rainfall manipulation experiments. The cages with different color indicated four treatments (white: natural rainfall, red: rainfall exclusion, green: double rainfall, and blue: half rainfall). Each column of cages contained single species.

(Pennings et al., 2005; Maestre et al., 2009) are caused by saline and low soil moisture. Soil salinity and moisture are regulated by the tidal inundation, runoff, and rainfall (Callaway and Sabraw, 1994; Omer, 2004; Habib et al., 2008; Levine et al., 2011), so the factors are uneven among different marsh zones and fluctuate with rainfall events. Seedling establishment is generally limited in the dry season when soils are hypersaline and soil moisture is low. Post-dry season rainfall lowers soil salinity and raises soil moisture, relieving stress for germination and seedling survival (Callaway et al., 1990; NOE and Zedler, 2001). Fluctuations in soil salinity and moisture, resulting from changes in rainfall patterns and tidal elevations, provide an opportunity for seedling establishment of different species. However, few studies have extended the discussion of differences in seedling emergence among tidal elevations after the dry season (Knapp et al., 2008; Levine et al., 2008; Levine et al., 2011), and we know little about how tidal inundation regulates the responses of seedling establishment to rainfall.

Tidal inundation regulates the stress from soil salinity and moisture on seedling establishment of key species in tidal salt marshes (Davy et al., 2011; Levine et al., 2011). In tidal salt marshes, the establishment of seedlings are more drive by hydrodynamics in low marsh caused by frequent flooding. Higher marsh zones are less frequently inundated by tides compared with lower marsh areas (Omer, 2004). As a result of infrequent tide, the soil surface in the higher marsh zones is prone to extreme saline and lower moisture conditions (Callaway and Sabraw,

1994). Seedling establishment of some species is limited by the harsher stress conditions of soil salinity and moisture in higher zones than lower marsh areas.

The success of seedling establishment depends on whether the changed soil conditions meet the threshold of seed germination and seedling emergence niches (Gomez-Aparicio et al., 2005; Soberon, 2007), which to a great extent are controlled by rainfall amount (Smith et al., 2000). In addition, the different species have differing niches to soil salinity and moisture, so species composition in the seedling life stage depends on post-dry season rainfall in drier and wetter years. Environment with high inter-annual rainfall variability are often only suitable for seedling niches in wetter years, however, the response of levels of seedling establishment on variability in post-dry rainfall has received relatively little attention. Seedling establishment studies conducted across a range of rainfall levels after the dry season have greatly improved our understanding of how rainfall events influence plant species persistence (Knapp et al., 2008).

To confirm whether the post-dry season rainfall provides the same opportunities for seedling establishment of plant species in different tidal elevations and in drier and wetter years with different rainfall regimes. We conducted a series of rainfall-manipulated experiments in salt marshes associated with controlled greenhouse experiments in 2015. The field experiments were located in National Nature Reserve salt marshes in Yellow River Delta, China. These salt marshes are ideal for experimentally testing this question because (He et al., 2017): 1) they contain the majority of the remaining native salt marshes in temperate China with lower anthropogenic stress (e.g. coastal development), and 2) plants dominating these salt marshes are commonly annual species (He et al., 2012a). In temperate China, the winter (December to February) and the early spring (early March) is the dry season, so the post dry rainfall is the spring rainfall. We controlled rainfall as the single variable in greenhouse experiments to simulate dry or wet years. Through controlled greenhouse experiments, we explored the seedling establishment response of different species under different stresses of soil salinity and moisture to rainfall variability.

We hypothesized that: (1) rainfall provides an opportunity for seedling establishment by strongly influencing soil salinity and moisture over the short term; (2) the effect of rainfall in promoting seedling establishment is associated with tidal inundation; and (3) rainfall variation interacts with niche modeling of different species depending on elevations.

## 2. Methods and materials

### 2.1. Study sites

We conducted experiments in a tidal marsh located in the core zone of the Yellow River Delta National Nature Reserve at Bohai Bay, northern China (118°68′E–119°34′E, 37°77′N–38°12′N) (Fig. 1 a) (He et al., 2012). Average annual rainfall was 559.1 ± 40.4 mm (mean ± SE) during 2000–2015, as recorded by the China's National Ground Meteorological Station (Kenli weather station, 118°33′E, 37°35′N). Rainfall was relatively seasonal with considerable monthly variability. The temperature became warm from March. January to March is the dry season in the Yellow River Delta and spring rainfall occurs in April and May (Fig. 1 b). Maximum spring rainfall was 130.3 mm and the minimum spring rainfall was 29.4 mm from 2000 to 2015. The variation of spring temperature is low during 2000–2015, but there are many severe droughts in the period (2000, 2001 and 2011) (Fig. 1 c). To examine the effect of spring rainfall on seedling establishment of key salt marsh species, our field study was conducted during the period from late March to May in 2015.

### 2.2. Tidal range data

Since the tidal elevation can influence seedling establishment in tidal marshes, we investigated the interaction between tidal elevation and rainfall events. The study region experiences irregular, semi-diurnal tides. To identify the effects of rainfall on seedling establishment under variable tides, we assigned study sites into different tidal zones identified by tide levels data. Tide levels were estimated based on the astronomic tide model at the Dongyinggang tidal station (118°58′E, 38°06′N), cited from the National Marine Data and Information Service (NMDIS), China. The lowest average tide (LAT), mean high water neap (MHWN), mean high water spring tide (MHWS), and highest average tide (HAT) were −0.74, −0.07, 0.34, and 0.63 m, respectively (Fig. 1d). The subtidal, low tidal, mid tidal, high tide, and supratidal zones were divided by the four tidal range lines (LAT, MHWN, MHWS, and HAT). Ten locations were randomly chosen along a sea-to-land gradient from the mid tidal to supratidal zone, avoiding the low tidal and subtidal zone with frequent tidal flooding. The elevation of the 10 locations, relative to sea level (Yellow Sea Datum, YSD), was measured using a GPS instrument (4600LS, Trimble GPS, Sunnyvale, California, USA). Two locations were located in the mid tidal zone (M1: 0.10 m, M2: 0.22 m), four locations were established in the high tide zone (H3: 0.40 m, H4: 0.46 m, H5: 0.52 m, H6: 0.58 m), and the other four study locations were located in the supratidal zone (S7: 0.72 m, S8: 0.78 m, S9: 1.00 m, S10: 1.44 m) (Table 1 and Fig. 2). We estimated flooding frequency for each zone based on long-term field observations (He et al., 2012b).

### 2.3. Post-rainfall investigation of soil factors dynamics

After the 2015 spring rainfall events, we investigated soil salinity and moisture content dynamics to test the hypothesis that fluctuations in soil moisture and salinity result from changes in rainfall patterns and tidal elevations. We randomly established three 1 m × 1 m quadrats in each of the 10 study locations. Soil cores (5 cm in diameter, 5 cm in depth) in each quadrat were sampled to determine soil salinity and moisture content at 0, 1, 2, 4, 8, 14, 22, 30, 38, 46, 54, 62, and 70 h after spring rainfall events. We completed post-rainfall soil factors investigations from later March to early May in 2015 (2 April, 19 April and 9 May). Soil salinity were measured using the rehydration method (Cui et al., 2011; Pennings et al., 2003). Soil cores were weighed and dried in an oven (60 °C, 48 h), and reweighed to determine moisture content.

### 2.4. Plant species

The harsh salinity conditions and tidal waves on the tidal flats are usually accompanied with low vegetation species diversity. *Suaeda salsa* is widely tolerant of salinity and flooding stresses, dominating most of the coastal marsh in the Yellow River Delta, except the supratidal

**Table 1**
Study locations and flooding in different tidal zones at the salt marsh.

| Tidal zone | Range | Location | Elevation | Flooding frequency* |
|---|---|---|---|---|
| Subtidal zone | < −0.74 m | – | – | 100% |
| Low tidal zone | −0.74 m to −0.07 m | – | – | 80–90% |
| Mid tidal zone | −0.07 m to 0.34 m | M1 | 0.10 m | 40–60% |
| | | M2 | 0.22 m | |
| High tide zone | 0.34 m to 0.63 m | H3 | 0.40 m | 5–15% |
| | | H4 | 0.46 m | |
| | | H5 | 0.52 m | |
| | | H6 | 0.58 m | |
| Supratidal zone | > 0.63 m | S7 | 0.72 m | 1% |
| | | S8 | 0.78 m | |
| | | S9 | 1.00 m | |
| | | S10 | 1.44 m | |

* Flooding frequency indicates the percentage of days flooded in a year.

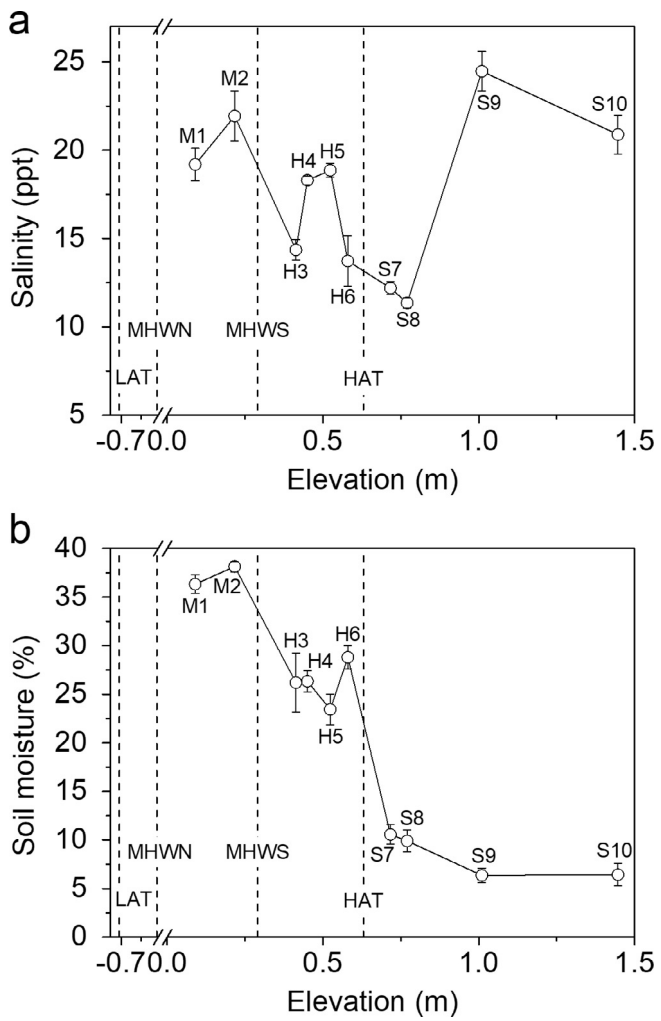


**Fig. 2.** (a) and (b) Soil salinity and moisture of field study locations during March to May in 2015. These locations were arranged at different tidal zones according to tide levels data and elevation of locations. The levels of lowest average tide (LAT), mean high water neap (MHWN), mean high water spring tide (MHWS), and highest average tide (HAT) were –0.74, –0.07, 0.34, and 0.63 m, respectively.

uplands (He et al., 2012). The high marsh zone is primarily covered by *S. salsa*, with interspersed patches of the halophyte *Salicornia europaea* (He et al., 2015). *Phragmites australis*, *Cynanchum chinense*, *Apocynum venetum* inhibit in supratidal zone (He et al., 2012; Li et al., 2016a,b) and their seeds are common in tidal marshes by wind-vector long distance dispersal.

*S. salsa, S. europaea, C. chinense*, and *A. venetum* were annual species. *Spartina alterniflora* is a newly invasive species that has colonized the low coastal marsh by seeds since 2012 and is spreading to the middle marsh. The pseudohalophyte species *P. australis* spreads to middle marsh by wind-vector seeds. The six species can be considered as planting in our study tidal marshes by seeds.

The seeds of *S. salsa, S. alterniflora, S. europaea, P. australis, C. chinense*, and *A. venetum* were collected in November 2014. These seeds were stored at −5 °C before use in the field and greenhouse experiments.

### 2.5. Rainfall manipulation experiments design

To test the hypothesis that rainfall associated with the tidal elevations provides an opportunity for seedling establishment in salt marshes, we conducted controlled field experiments in five out of the 10 study locations (M1, H3, H5, S8, and S9) along an elevation gradient across three tidal zones. We collected surface soil (0–5 cm) at the five study locations in October 2014 before the seed production period (from later October to December) to ensure no active seeds in soil. The soil was dried at natural temperature for two months and then stored at −5 °C. Then, the soil samples from each site were filled into plastic pots of 30 cm in diameter and 20 cm depth for use in the field experiment. Mature seeds collected from the six species were distributed in pots at 1 cm in depth. Each pot contained seeds from one species to avoid interspecies competition in the seedlings stage (150 seeds of *S. salsa*, 100 seeds of *S. europaea,* 50 seeds of *S. alterniflora*, 50 seeds of *C. chinense*, 100 seeds of *A. venetum*, or 50 seeds of *P. australis*). 180 pots have been prepared for each species.

We established 12 plots (2 m × 2 m) in each of the five study locations in March 2015 for a total of 60 plots, six of which were randomly assigned to each of the four treatments including natural rainfall, rainfall exclusion, double rainfall, and half rainfall in the field experiment (Smith, 2011a,b; Goldstein and Suding, 2014). In every 2 m × 2 m plot, 3 pots for each species and total 18 pots were arranged and spaced 0.5 m apart. There was no slope, minimizing runoff from rain events. All of the pots were inserted into the soil and the edge of the pot was levelled with the ground surface. Every pot was caged with galvanised hardware mesh (1.0 cm mesh size, 35 cm diameter, 40 and 10 cm above and belowground, respectively) held by wooden stakes to limit herbivory from crabs in the Yellow River Delta (He et al., 2017). Plastic mesh (0.7 mm mesh, smaller than most seeds of the six species) was placed outside the hardware mesh to isolate pots from external seeds and small animal herbivory (Fig. 1e).

Rainfall treatment plots included pots of six species with two-layer mesh without rainfall-proof manipulation. The rainfall exclusion treatment was constructed using a transparent waterproof cloth that was tied to the outside of the plastic mesh with openings (10 cm high) at the soil surface to allow tidal water access and to maintain inside and outside temperature. The double rainfall treatment had the same design cages of the rainfall exclusion treatment, but freshwater was supplemented to double the volume (100–700 mL) after every rainfall event using an injection syringe to penetrate the waterproof cloth. The half rainfall treatment had the same design as the rainfall exclusion and double rainfall treatment and was supplemented with freshwater with half the volume (30–150 mL) after every rainfall event. Treatment manipulations were maintained weekly as needed. Thereafter, we recorded seedling numbers every week from March to May 2015.

### 2.6. The greenhouse simulation experiments

To examine how soil conditions (controlled by the tidal elevation) influenced the effect of rainfall events on seedling establishment and to identify the response of seedlings to extreme spring rainfall, we expanded the field manipulating experiments into more scenarios. We conducted a greenhouse experiment to simulate seedling establishment under stress gradients (salinity and moisture) to freshwater introduction (instead of rainfall). The stress gradients were confirmed based on post-rainfall investigation of soil factors dynamics. The gradient of salinity was 8, 12, 16, 20, or 24 parts per thousand (ppt) and 5%, 10%, 16%, 24%, or 30% soil moisture. The testing gradients were choosing based on the natural range of soil salinity and moisture conditions in the study sites (Xie et al., 2018). We assigned pots into 25 stressed scenarios under salinity and moisture gradient recordings (8 ppt – 5%, 8 ppt – 10%, 8 ppt – 16%, 8 ppt – 24%, 8 ppt – 30%, 12 ppt – 5%, 12 ppt – 10%, 12 ppt – 16%, 12 ppt – 24%, 12 ppt – 30%, 16 ppt – 5%, 16 ppt – 10%, 16 ppt – 16%, 16 ppt – 24%, 16 ppt – 30%, 20 ppt – 5%, 20 ppt – 10%, 20 ppt – 16%, 20 ppt – 24%, 20 ppt – 30%, 24 ppt – 5%, 24 ppt – 10%, 24 ppt – 16%, 24 ppt – 24%, 24 ppt – 30%). We used 8, 12, 16, 20, or 24 ppt saline water to maintain the salinity gradient, and supplemented the saline water in these pots with 250 mL every day to offset evaporation, which kept the soil moisture at about 30%, 24%, 16%,

10%, or 5%, respectively. The greenhouse pots with seeds were of the same design as the rainfall manipulation experiments described above.

In every scenario, we established three treatments: blank control, large rainfall, and small rainfall. In the blank control treatments, seeds of six species were cultivated under stress scenarios for two months and the salinity and moisture conditions were maintained the whole time. In the large rainfall treatment, total rainfall was 130 mm to simulate an extremely wet spring, according to rainfall data from 2000 to 2015. The small rainfall treatment received 30 mm rainfall, which simulated an extremely dry spring. We controlled the amount of rainfall, adding freshwater with a drip system at a rate of 5 mm/h in greenhouse (Goldstein and Suding, 2014). We simulated rainfall on six different dates in two month-long greenhouse experiments and every rainfall event lasted four hours in the large rainfall treatment and one hour in the small rainfall treatment.

All of the treatments were replicated six times in a greenhouse, so we used 2700 pots (3 (treatments) × 6 (replications) × 6 (species) × 25 (scenarios)) in total for this experiment. In the greenhouse, supplementary light was available between 06:00 and 18:00 and the temperature was maintained at 20 °C (06:00–18:00) or 10 °C (18:00–06:00), which is the spring temperature in the Yellow River Delta. Seedling emergence was recorded every day.

### 2.7. Data analysis

We used ANOVAs with Tukey post hoc multiple tests to examine differences among all treatments (including control and exclusion control treatments) at each site. We used full factorial repeated measures ANOVAs with Tukey post hoc multiple tests to test for differences in the salinity gradient, soil moisture gradient, and rainfall treatments. All analyses were done with SPSS 20 software (International Business Machine Corporation). Data sets were natural-log transformed when necessary to meet assumptions of normality and homogeneity of variance.

In order to further compare the results of field rainfall manipulation experiments and greenhouse simulation experiments, a response surface analysis (RSA) (Law and Watkinson, 1987) was used to identify the relationship of seedling emergence with two variables (soil salinity and moisture). We conducted contour analysis (CA) based on RSA to confirm the value of salinity and moisture when seedling emergence was 5%, which could be considered as the minimum credible threshold for seedling establishment in consideration of the absence of an Allee effect (Deredec and Courchamp, 2007). The soil salinity and moisture of field sites in field manipulation experiments were compared to the results of the contour analysis. The RSA and CA were conducted with MATLAB 2012b (Apple Hill Drive, Natick, MA, USA) and Origin 8.5 (OriginLab Corporation, Northampton, MA, USA).

## 3. Results

### 3.1. Post-rainfall fluctuation of soil salinity and moisture

The temperature became warm from March and the average spring temperature was 14.7 ± 0.7 °C in year 2015 (Fig. 3 a). There were 9 times rainfall events more than 10 mm in spring 2015 (Fig. 3 a). After rainfall events, soil salinity decreased ($F_{1, 30} = 72.672$, $P < 0.001$) and soil moisture rose ($F_{1, 30} = 10.769$, $P = 0.002$) (Fig. 3b and c). The largest changes in soil salinity occurred in high tidal zone (H4 and H5), and the most intensive decrease in soil moisture was in supratidal zone (S9 and S10). The fluctuation of soil salinity ($F_{12,30} = 147.135$, $P < 0.001$) and moisture ($F_{12, 30} = 419.202$, $P < 0.001$) was significantly associated with time after the rainfall event. The duration of fluctuation of soil salinity at H5, H6, S7, S8, S9, and S10 was more than 70 h. At locations M1, M2, H3, and H4, the duration was about 46 to 62 h after the rainfall event. Soil moisture decreased to initial drier conditions 46–70 h after rainfall events in the middle and high tidal zone. However, the higher moisture caused by rainfall only remained 38 h at locations S8, S9, and S10 in the supratidal zone.

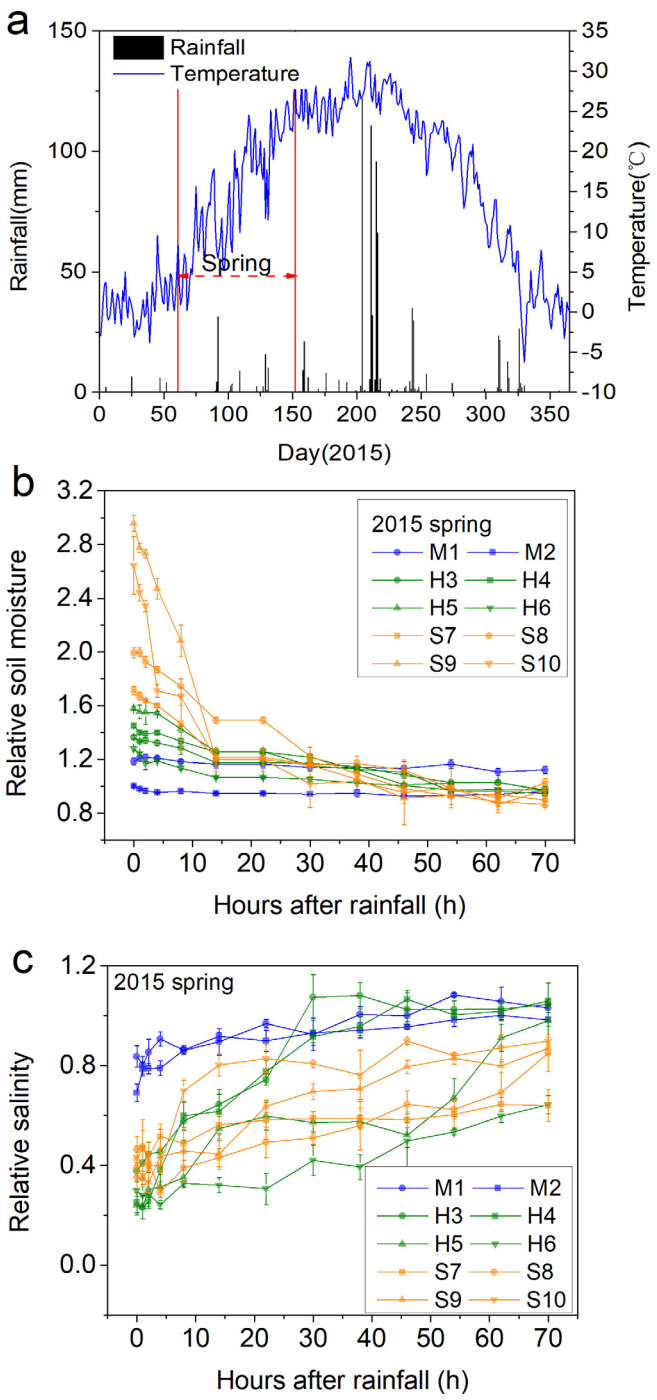


**Fig. 3.** (a) The daily rainfall and temperature data set in 2015. (b) and (c) the fluctuation of soil moisture and salinity after rainfall at 10 study locations during spring 2015. The relative moisture and relative salinity were the ratio between observed moisture and salinity after rainfall with the soil moisture and salinity gradients of 10 locations, respectively.

### 3.2. Seedling establishment response to spring rainfall and the tidal regime

The influence of spring rainfall on seedling establishment was different across tidal zone and species. The seedling establishment of the six species is more dependent on rainfall amount in mid-tidal zone and supratidal zone than high tidal zone. *S. salsa* and *S. europaea* seedling

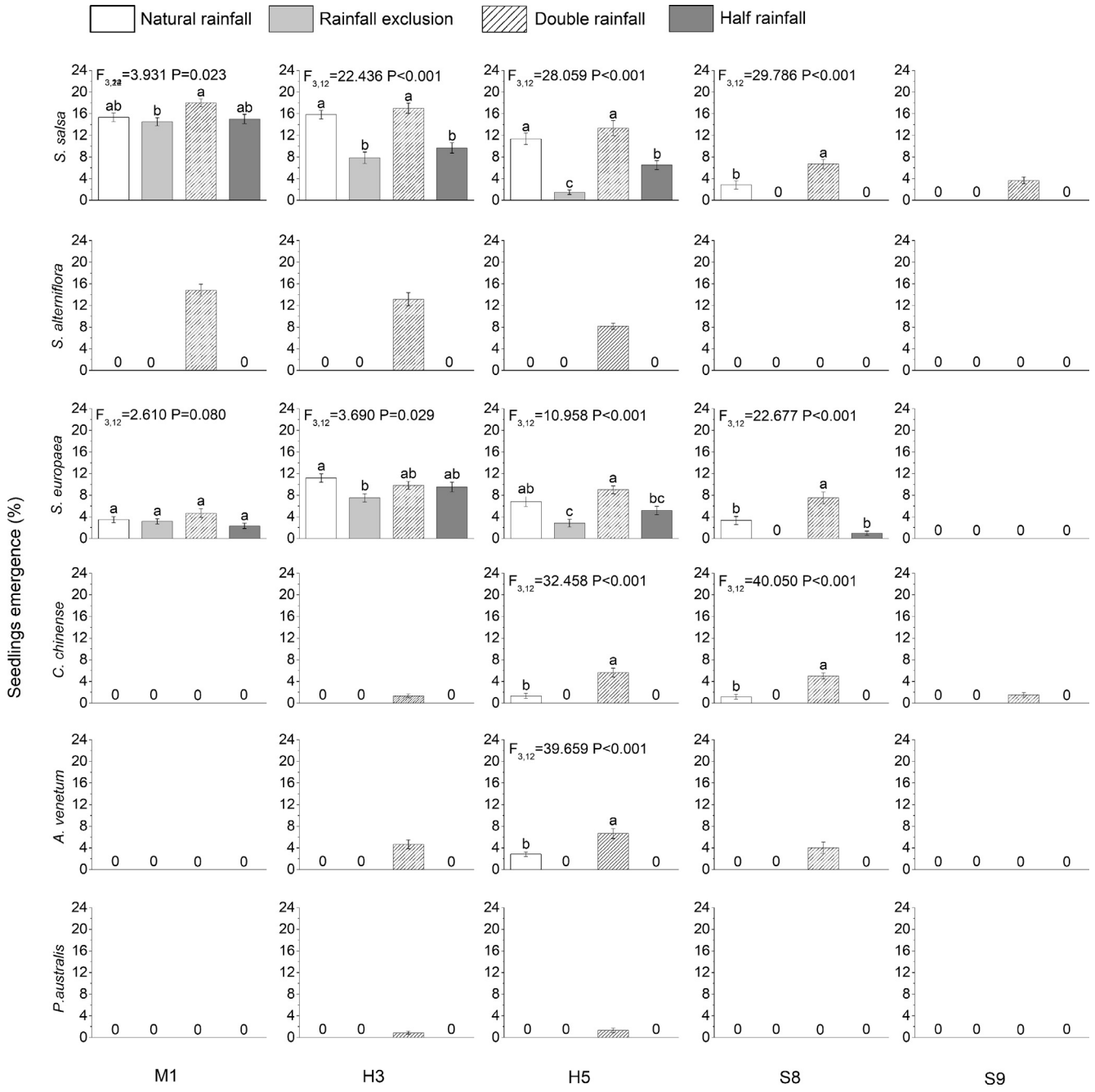


**Fig. 4.** Seedling emergence of six species in four rainfall manipulation treatments at five study locations. Data are shown as means ± SE. Letters indicate significant differences within a rainfall treatment, with a Tukey test.

emergence did not differ between the natural rainfall, rainfall-exclusion, half rainfall and double rainfall treatments in the mid-tidal zone but was significantly in the high tidal zone. Seedling emergence for *S. salsa* and *S. europaea* was only success in the natural rainfall and double treatments in the supratidal zone, and the seedling establishment in the double rainfall treatment was significantly higher. No seedlings of *S. alterniflora*, *C. chinense*, *A. venetum*, and *P. australis* were recorded no matter whether rainfall was excluded or not in the mid-tidal and the high zone. The double rainfall treatment achieved significantly better performance for *S. alterniflora*, *C. chinense*, *A. venetum*, and *P. australis* in the high tidal zone, but seedling establishment of *S. alterniflora, P. australis* and *A. venetum* failed in all treatments in the supratidal zone (Fig. 4).

### 3.3. Seedling establishment of different species response to soil conditions

In the greenhouse, seedlings emergence of the six species was negatively correlated with soil salinity and positively related with the moisture gradient (Fig. 5). The simulated rainfall improved seedling emergence of halophytes (*S. salsa*, *S. alterniflora* and *S. europaea*) and a pseudohalophyte (*P. australis*) at a greater range of soil salinity than non-halophytes (*C. chinense* and *A. venetum*).

The effect of simulated rainfall on seedling emergence of halophytes and pseudohalophytes was significantly associated with soil salinity and soil moisture gradient. The simulated rainfall had little influence on seedling establishment of non-halophytes when salinity was more than 16 ppt. The species *C. chinense* and *A. venetum* are highly adaptable to soil moisture and the effect of rainfall on seedling establishment

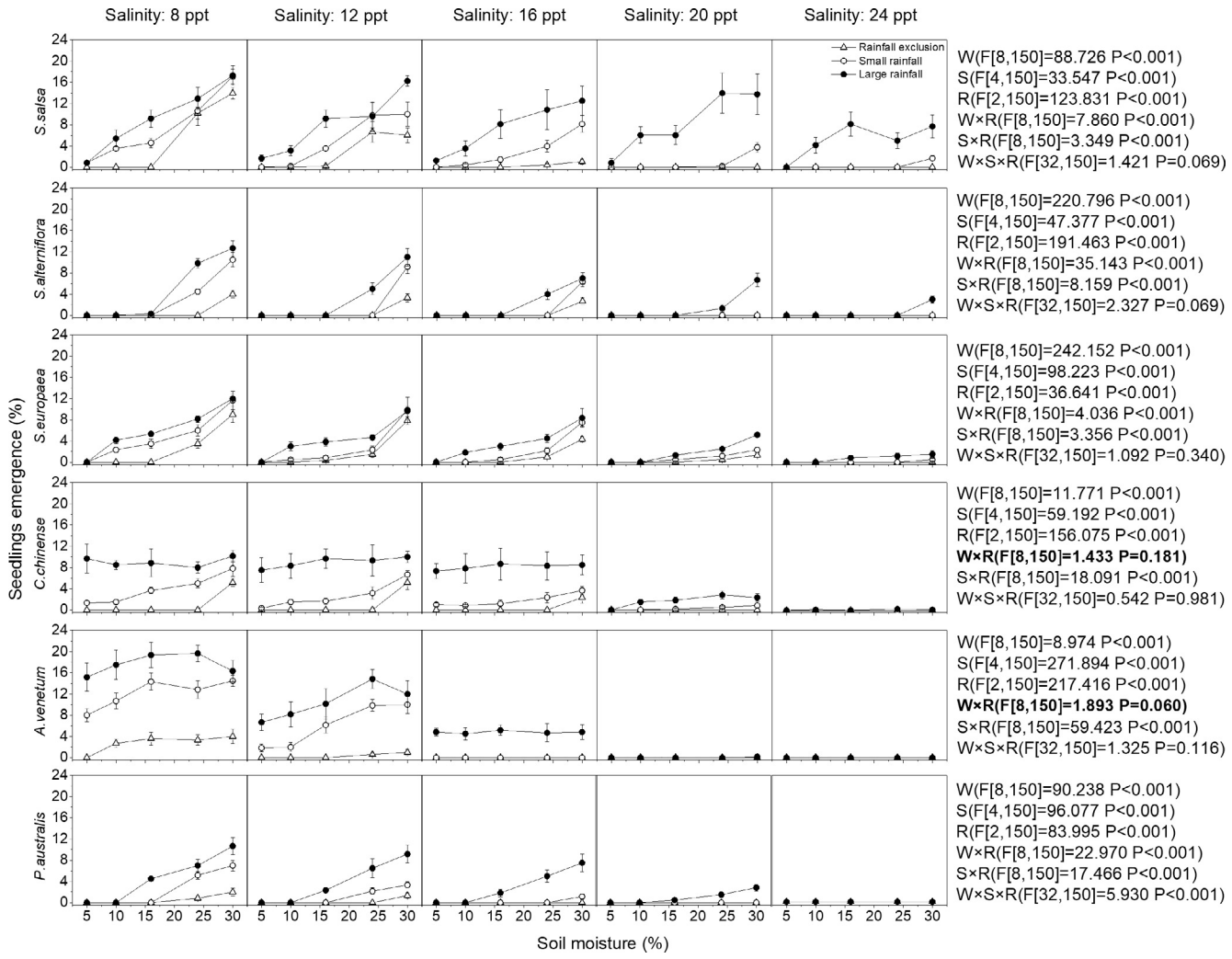


**Fig. 5.** Seedling emergence of six species under salinity and moisture gradients at three rainfall treatments in greenhouse experiments. Figures show means ± SE, with statistical parameters in the graph right edge. Abbreviations are: W, soil moisture gradient; S, soil salinity gradient; R, rainfall treatment.

differed insignificantly along the soil moisture gradient.

### 3.4. Seedling establishment under extreme spring rainfall

The differences of seedling establishment among the three rainfall amount simulation treatments, including rainfall exclusion, extremely small, and extremely large rainfall treatments, was significant for all six species (Fig. 5). Seedling establishment of *S. salsa*, *S. alterniflora*, *S. europaea* and *P. australis* in the extremely large simulated spring rainfall treatment was only significantly higher than the other two treatments in high salinity and moist conditions. The effect of the large rainfall treatments in promoting seedling establishment of *C. chinense* and *A. venetum* was significantly better than the small rainfall treatment along the entire moisture gradient and with 12 and 16 ppt soil salinity.

We used RSA to further explore the niche modeling to see which locations were expected to have variable seedling establishment due to rain. The contour line with 5% seedling emergence based on RSA indicated the minimum threshold for seedling establishment with extremely small rainfall amounts and extremely large rainfall amounts (Fig. 6). The soil salinity and moisture of the field locations were compared to the threshold lines for seedling establishment. The soil conditions of locations M1 and M2 were suitable for seedling establishment of all six species with both the extremely small or large spring rainfall. In contrast, spring rainfall failed to affect seedling establishment in locations S9 and S10 due to hypersaline and dry soil conditions. Seedling re-establishment in other locations depended on the species and spring rainfall amount.

## 4. Discussion

Understanding the influence of spring rainfall on seedling establishment is critical for predicting the ecological consequences of potential changes to the rainfall regime. Perhaps the most important finding of this study was that manipulation of rainfall at the beginning of the growing season could control plant–soil interactions (Yando et al., 2016). Our study confirmed that rainfall provided an opportunity for seedling establishment by strongly influencing soil salinity and moisture to meet the seedling niche of plant species over the short term. However, the mechanism underlying this response was different across tidal elevations and species. Individual species in the tidal marsh had different responses to the seedling establishment niche of soil salinity and moisture, and the soil factors depended on tides and were altered by rainfall (NOE and Zedler, 2001). Our results suggest that shifts in species composition would emerge in the seedling establishment stage in response to changing rainfall regimes (Jentsch et al., 2007).

### 4.1. Seedling establishment response to spring rainfall amounts

In agreement with results from other studies on salt marshes (Goldstein and Suding, 2014; van der Zee et al., 2014), we found that

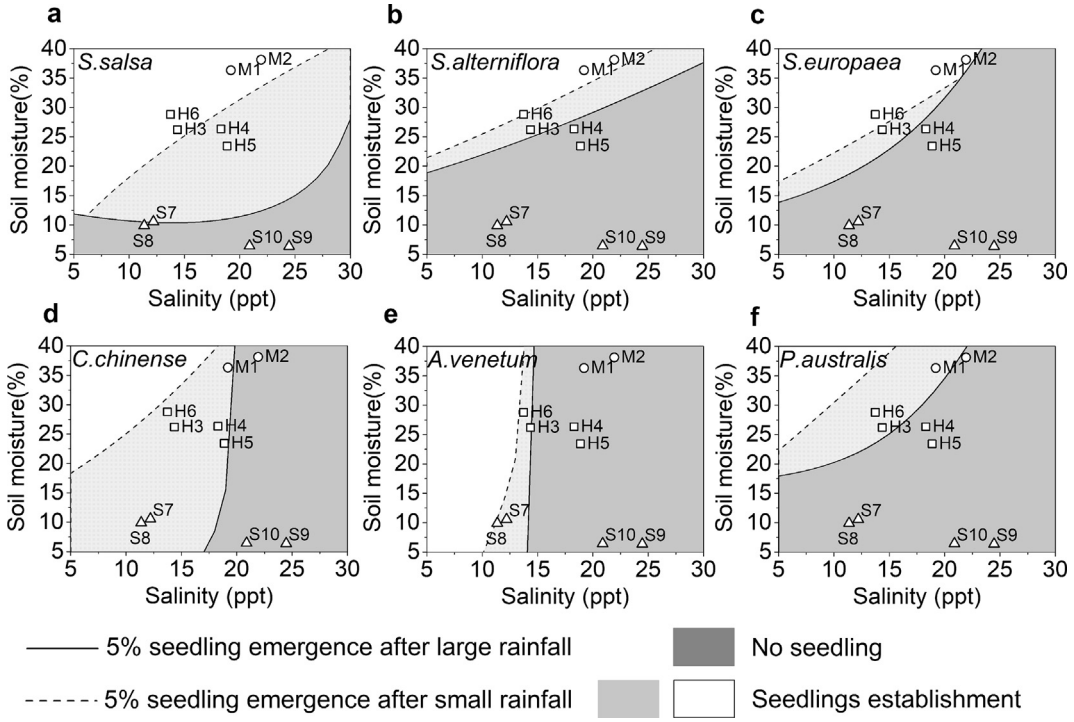


**Fig. 6.** Seedling emergence niche modeling of rainfall and tidal elevations. Contour analysis based on response surface analysis (RSA) confirming the value of salinity and moisture when seedling emergence was 5%. The dash line was the minimum credible threshold for seedling establishment under small rainfall treatments. The white above the dash line indicates seedlings established. The solid line was the minimum credible threshold for seedling establishment under large rainfall treatments ($R^2$ = 0.847, P = 0.000 of *S. salsa*, $R^2$ = 0.865, P = 0.000 of *S. alterniflora*, $R^2$ = 0.898, P = 0.000 of *S. europaea*, $R^2$ = 0.642, P = 0.000 of *P. australis*, $R^2$ = 0.945, P = 0.000 of *C. chinense*, $R^2$ = 0.919, P = 0.000 of *A. venetum*). The gray region above the solid line indicates seedlings established ($R^2$ = 0.850, P = 0.000 of *S. salsa*, $R^2$ = 0.819, P = 0.000 of *S. alterniflora*, $R^2$ = 0.926, P = 0.000 of *S. europaea*, $R^2$ = 0.904, P = 0.000 of *P. australis*, $R^2$ = 0.842, P = 0.000 of *C. chinense*, $R^2$ = 0.967, P = 0.000 of *A. venetum*) and the dark area represents the obstinate state. The gray region was no seedling establishment.

rainfall alters soil salinity and moisture over the short term. Our results showed soil salinity was reduced to half, and even a quarter of its initial level, and soil moisture was raised by 40% to 300% after a single post-dry season rainfall. The soil salinity and moisture returned to its original value 30–50 h later. In salt marshes, soil salinity and moisture were generally predominant factors controlling seedling establishment (Munns, 2002; Munns and Tester, 2008).

Seedlings would emerge after a few days in favorable soil environments when temperatures became warmer in the spring. In most areas of the marsh, however, saline and dry soil had much to do with the scarcity of rain and the dry air in winter. The soil salinity and moisture exceeded the tolerance thresholds of seeds and inhibited seedling establishment of some sensitive species. Post-dry season rainfall indeed provided a “window” for seedling establishment by lowering soil salinity and raising moisture for a short period (Balke et al., 2013).

The effect of rainfall was confirmed by the rainfall exclusion experiment design, and these findings are in agree with studies reporting that rainfall enhances plant population recruitment (Smith et al., 2000). The results of the rainfall manipulation experiments showed that seedling emergence was significantly affected by rainfall amount. The large rainfall treatments would cause a wide fluctuation in the range of soil salinity and moisture (Knapp et al., 2002; NOE and Zedler, 2001). In addition, the favored soil environment for seedling establishment was maintained for a longer time after a larger post-dry season rainfall. Our results also suggested that seedling establishment is highly dependent on rainfall amount, even for drought-tolerant and halophyte species. Actually, seedlings of species that are sensitive to saline soil or have low tolerance threshold to soil moisture, such as *C. chinense*, *A. venetum*, and *S. alterniflora* only established successfully during large rainfall events in our experiments.

### 4.2. The effect of rainfall associated with tidal elevations

The abiotic stress gradients from soil conditions on seed germination and seedling emergence was different across tidal elevations (Watson and Byrne, 2009; Davy et al., 2011; Levine et al, 2011). The soil salinity and moisture of tidal marshes is highly dependent on the frequency of tidal inundations (Omer, 2004), and normally soil exposure to frequent tides results in high moisture and stable salinity (Callaway and Sabraw, 1994). In our study region, the mid-tidal zone is wetter and less saline than the high tidal or supratidal zones. Our field experiments and greenhouse simulation experiments collectively showed the soil salinity and moisture in the mid tidal zone was suitable for seedling emergence of halophyte species, such as *S. salsa*. Halophyte species were able to establish seedlings at mid tidal zone even in the rainfall exclusion treatment. However, the soil salinity and moisture in high tidal or supratidal zones during the beginning of spring inhibited seedling establishment of plant species. Following post-dry season rainfall, marsh regions are subject to lower soil salinities and higher moisture, which can relieve the stress (Goldstein and Suding, 2014; van der Zee et al., 2014). A significant fluctuation of soil salinity and moisture was observed after rainfall in high tidal and supratidal zones where infrequent tidal inundations resulted in hypersaline and dry soil conditions; nevertheless, soil salinity and moisture changed gently in the mid tidal zone. In addition, the suitability for seedling establishment niches depended on soil conditions gradient (Zimmermann et al., 2008). The regions with extremely high salinity or dry soil, usually observed in high tidal or supratidal zones, could not reach the seedling niches even after spring rainfall. Therefore, our study showed the effect of rainfall on seedling establishment had a nonlinear relationship with abiotic gradients controlled by tidal elevations.

### 4.3. Alternative state and niche modeling

Our results found which locations in salt marsh were expected to have variable seedling establishment due to rain, and implied some locations were in an alternative state in which rainfall amount marked a seedling establishing niche between two opposing stable ecosystem states, e.g., seedling establishment and bare flats (Friess et al., 2012; Silinski et al., 2015).

In our study, *C. chinense* and *A. venetum* were drought-tolerant species that were sensitive to soil salinity. The high salinity locations M2, S9, and S10 of *C. chinense* and M1, M2, H4, H5, S9, and S10 of *A. venetum* were obstinate to rainfall for seedling establishment. The locations H3, H6, S7, and S8 (H4 and H5 for *C. chinense*) were in an alternative state and the seedlings of *C. chinense* and *A. venetum* only emerged after large rainfall amounts. Our results showed the seedlings of *C. chinense* and *A. venetum* disappeared entirely in these areas of the marsh in dry years with little spring rainfall. The persistence of species *C. chinense* and *A. venetum* in our study region was especially dependent on spring rainfall (Smith et al., 2016).

The halophyte species *S. salsa*, *S. alterniflora*, and *S. europaea*, have a high tolerance for soil salinity (Pennings et al., 2005, He et al., 2015). These species freely established their seedlings when temperatures became warm (more than 10 °C, Fig. 1 b) in the mid tidal locations (H1, H2) and high tidal location with available soil moisture (H6). However, according to our greenhouse experiments results, the soil salinity and moisture at supratidal locations (S7, S8, S9, and S10) exceeded the seedling emergence niches of three halophyte species even under the effect of large rainfall events. The seedling emergence of species *S. alterniflora* had higher demand for soil moisture, so some high tidal locations (H4, H5) with dry soil were still not suitable for seedling establishment niche after large rainfall events. For *S. salsa*, seedling establishment at high tidal locations H4 and H5 was successful under large rainfall amounts but was invalid in small rainfall events. Similarly, the rainfall amount determined whether *S. alterniflora* seedlings emerged at H3 and H6 and whether *S. europaea* emerged at H3.

### 4.4. Species composition changes and invasion

The rainfall manipulation experiments allowed us to test the response of species to changes in spring rainfall. Our results provide a foundation for better understanding how dynamic abiotic conditions limit seedling establishment of species that can survive and become dominant in salt marshes. The plant–soil–rainfall interactions were nonlinear across soil conditions gradient controlled by tidal elevations. The tidal zone in alternative states were instable where small changes in spring rainfall could trigger comparatively large and abrupt changes in seedling establishment of different species. In dry years with small spring rainfall amounts, *S. salsa* at high tidal locations (H4 and H5) failed to establish seedlings and the supratidal locations shifted into bare flats. However, the invasive species *S. alterniflora* expanded into the high tidal zone under large spring rainfall amounts, if taking no account of dispersal limitations. From a community perspective, species composition of seedlings was generally located in tidal zones where small differences in abiotic conditions resulted in comparatively large differences in seedling establishment of key species (Goldstein and Suding, 2014; Osland et al., 2016).

Our results suggest that rainfall changes could alter invasion dynamics in the seedling establishment stage (Oconnor, 1995). It has been hypothesized that invasive species are inhibited by water limitation and take advantage of high resource availability during rainfall pulses (Davis et al., 2000; Hulme, 2005). We simulated a post-rainfall species composition of native seedlings and invasive species *S. alterniflora*, and found that potential rainfall changes with larger rainfall amounts may increase invasion. The rainfall regime changes may cause shifts within the native species composition as well and open invasion opportunities to new species in response to salinity or moisture stress (Soberon, 2007).

### 4.5. Implications for the effects of future climate change

Due to their position at the land–sea interface, coastal wetlands are vulnerable to many aspects of climate change. Tidal inundation likely has the largest impact on coastal wetlands because flooding and salinity regimes are tremendously important abiotic drivers within these systems. However, rainfall events drive the fluctuation of salinity and moisture across different tidal zones, and the rainfall effect is too important to ignore. This study advanced our understanding that rainfall variation interacting with abiotic gradients which regulated by tidal inundation influenced suitability of abiotic factors for ecological niches of different species in salt marshes.

We concluded changing rainfall regimes could lead to changes in the composition of key plant species within ecosystems (Callaway and Sabraw, 1994). Incorporating the ecological consequences of rainfall changing into current conservation efforts is an increasingly critical challenge for ecologists and natural resource managers (Levine et al., 2011; Osland et al., 2016). We recommend that interactions between rainfall and abiotic linkages and species niche should be studied more intensively so that we can better understand and forecast the effects of changing rainfall regimes (Smith, 2011a,b; Osland et al., 2014). In addition, our results have implications for coastal wetland management and restoration efforts, which should consider and prepare for the effects of future rainfall changes related to climate change.

## 5. Authorship

TX, BC, and SL designed study; TX, SL, QW and WS performed field and greenhouse experiment; TX and SL analyzed data; TX, BC and JB wrote the manuscript, and all authors contributed substantially to revisions.

## Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this paper.

## Acknowledgments

We thank Steven C. Pennings for helpful comments on the manuscript. The study was supported financially by Key Project of National Natural Science Foundation of China (51639001), Project supported by the Fund for Innovative Research Group of the National Natural Science Foundation of China (51721093) and National Key Basic Research Program of China (2013CB430406).

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